AD NUMBER

AD477355

NEW LIMITATION CHANGE

TO

Approved for public release, distribution unlimited

FROM

Distribution authorized to U.S. Gov't. agencies and their contractors; Administrative/Operational Use; Jan 1966. Other requests shall be referred to US Army Biological Laboratories, Fort Detrick, MD 21701.

AUTHORITY

BORL D/A ltr, 28 Sep 1971

AD



TECHNICAL MANUSCRIPT 256

# SOME CHARACTERISTICS AND KINETICS OF COAGULASE RELEASE BY STAPHYLOCOCCUS AUREUS

Robert A. Altenbern

JANUARY 1966



UNITED STATES ARMY
BIOLOGICAL LABORATORIES
FORT DETRICK

## DDC AVAILABILITY NOTICES

Qualified requestors may obtain copies of this publication from DDC.

Foreign announcement and dissemination of this publication by DDC is not authorized.

Release or announcement to the public is not authorized.

## DISPOSITION INSTRUCTIONS

Destroy this publication when it is no longer needed. Do not return it to the originator.

The findings in this publication are not to be construed as an official Department of the Army position, unless so designated by other authorized documents.

U.S. ARMY BIOLOGICAL LABORATORIES
Fort Detrick, Frederick, Maryland

TECHNICAL MANUSCRIPT 256

# SOME CHARACTERISTICS AND KINETICS OF COAGULASE RELEASE BY STAPHYLOCOCCUS AUREUS

Robert A. Altenbern

Medical Bacteriology Division
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1C014501B71A01

January 1966

## ABSTRACT

Coagulase is released from growing cells of strains of Staphylococcus aureus when purified bovine serum albumin is added to the medium. When cells are grown in albumin-free medium to an optical density of 0.2 and then resuspended in media containing albumin, two types of responses are noted: some strains show an immediate and steady rate of release of coagulase, others show a considerable (40 to 60 min) lag followed by release of coagulase at a much lower but also steady rate. Coagulase is not released by resuspension in albumin solution alone. Horse, human, bovine, and porcine albumins elicit the same rate of coagulase release, but egg albumin promotes coagulase release at a considerably lower rate. Bovine hemoglobin, gelatin, transferrin, and bovine alpha, beta, and gamma globulins do not promote the release of coagulase. Slightly acid pH suppresses release of coagulase from cells growing in media containing albumin. Proper concentrations of chloramphenicol completely prevent elaboration of coagulase but allow unaltered cell growth. Cell-free extracts prepared by two methods failed to show any intracellular coagulase.

# SOME CHARACTERISTICS AND KINETICS OF COAGULASE RELEASE BY STAPHYLOCOCCUS AUREUS

Although staphylococcal coagulase has been extensively studied, including purification of the active material and definition of the mechanism of action on fibrinogen, there are very few reports concerning the kinetics of formation and release of this substance from staphylococcal cells. Current status of research on coagulase has been recently summarized by Tager and Drummond.* This presentation will offer data on the characteristics and kinetics of release of coagulase by staphylococcal cells growing in medium containing mammalian albumin.

Various strains of Staphylococcus aureus isolated from clinical material submitted to the laboratory of the Frederick Memorial Hospital were employed. Stock cultures were maintained on trypticase soy agar slants. Synthesis of coagulase was studied in trypticase soy broth cultures supplemented with various proteins and incubated at 37 C on a shaker. Any modifications of this procedure will be described when appropriate. Coagulase was assayed in the following manner: The sample to be assayed was diluted serially in 1.5-fold dilution steps in 0.85% saline plus 50 µg chloramphenicol per ml so that 0.25 ml remained in each tube. To each tube was added 0.5 ml of substrate consisting of 1.5% bovine fibrinogen and 1.5% Warner-Chilcott standardized human plasma, all dissolved in saline-chloramphenicol diluent just described. After incubation at 37 C for 3 hours the highest dilution showing evidence of clotting was recorded and the reciprocal of this dilution represented the number of coagulase units per ml of the original sample.

All strains tested in this investigation failed to produce measurable coagulase during growth in trypticase soy broth. Inclusion of bovine serum albumin in varying amounts induced the release of coagulase, confirming earlier data published by Duthie** (1954). Figure 1 shows that two of the strains tested showed a regular increase in rate of coagulase release as the amount of albumin in the medium increased. The maximum rate of coagulase release was attained at an albumin concentration of about 0.15%, also confirming Duthie's** early papers. There was no immediately apparent explanation of the reason why albumin promoted coagulase release. It seemed highly unlikely that albumin was acting as a substrate, thus inducing the formation of coagulase. Several experiments showed that there was no precursor of coagulase elaborated during growth in albumin-free medium that could be activated by later addition of albumin.

---

* Tager, Morris; Drummond Margaret C. 1965. Staphylocoagulase. Ann. N.Y. Acad. Sci. 128:92-110.

** Duthie, E.S. 1954. The production of free staphylococcal coagulase. J. Gen. Microbiol. 10:437-444.

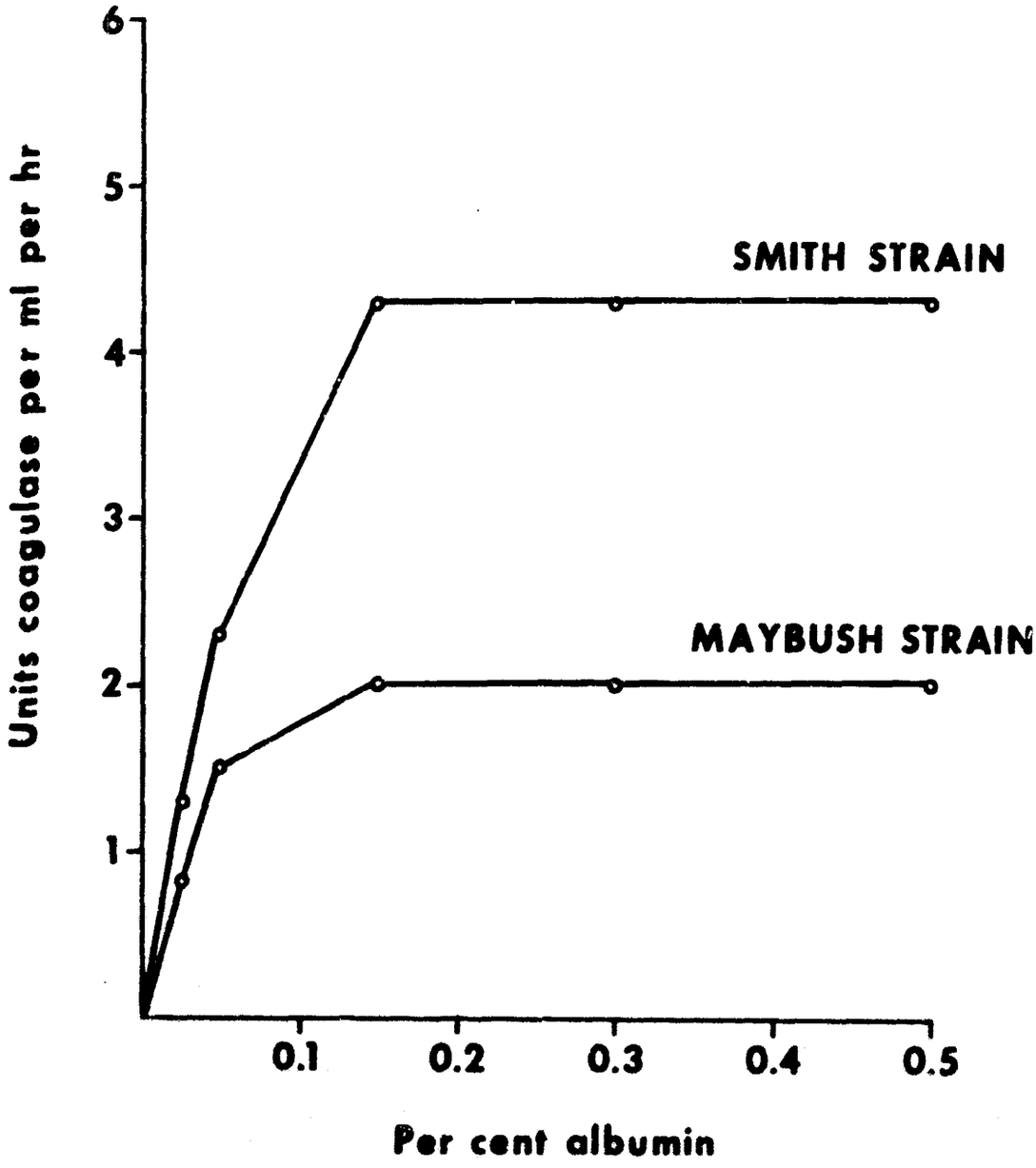


Figure 1. Rate of Coagulase Release of Staphylococcus aureus as a Function of Albumin Concentration.

Therefore, we studied the kinetics of coagulase release by cells grown to an optical density of 0.2 to 0.3 in albumin-free broth followed by resuspension in prewarmed broth containing 0.3% bovine albumin. Two types of responses were obtained (Fig. 2). One type exhibited an immediate coagulase release at a rate that was steady over at least 100 minutes, whereas the other type showed a considerable delay followed by coagulase release at a steady rate considerable less than the first type. In the case of the first type at least, the possibility of induction could be ruled out because there was clearly no inductive lag. The reasons for the lag period of the second type are obscure. It still seems unlikely that we are dealing with constitutive and inducible strains inasmuch as albumin bears no resemblance to fibrinogen, the substrate for coagulase. On the chance that impurities were present in the albumin that might trigger some sort of induction, crystalline bovine albumin was used and identical behavior of the strains was obtained. Since the growth medium was very rich nutritionally, it appeared unreasonable to assume that the albumins were providing some nutrient necessary for coagulase synthesis.

The efficacy of various albumins in promoting coagulase release was also examined. The data in Figure 3 show that, with strain Maybush, equine, bovine, porcine, and human albumins gave identical effects, but egg albumin promoted a rate of coagulase release significantly smaller than those from mammalian albumins. Other proteins were tested and the following were completely inactive: gelatin, bovine hemoglobin, transferrin, bovine alpha, beta, and gamma globulins. On this basis there seems to be some specific property of the albumin molecules that promotes coagulase release.

Several experiments have shown that bovine albumin does not release coagulase by a simple exchange reaction. Cells of the Maybush and Smith strains were grown in trypticase soy broth to an optical density of 0.2, centrifuged, resuspended in chilled (4 C) broth containing 0.3% bovine albumin, and stored in the refrigerator. There was no release of coagulase from these cells stored for as long as 24 hours. Similarly, cells grown in albumin-free broth and resuspended in 0.3% bovine albumin alone followed by incubation at 37 C allowed no coagulase release.

Subsequent experiments showed that release of coagulase during growth in albumin broth was completely prevented by chloramphenicol (Fig. 4). These results showed that in addition to elimination of exchange as a mechanism of release, active and continuing protein synthesis was necessary for elaboration of coagulase into the medium. Further studies on the effects of chloramphenicol (Table 1) revealed that complete inhibition of release of coagulase could be effected by chloramphenicol concentrations too small to have a detectable effect on the growth of the

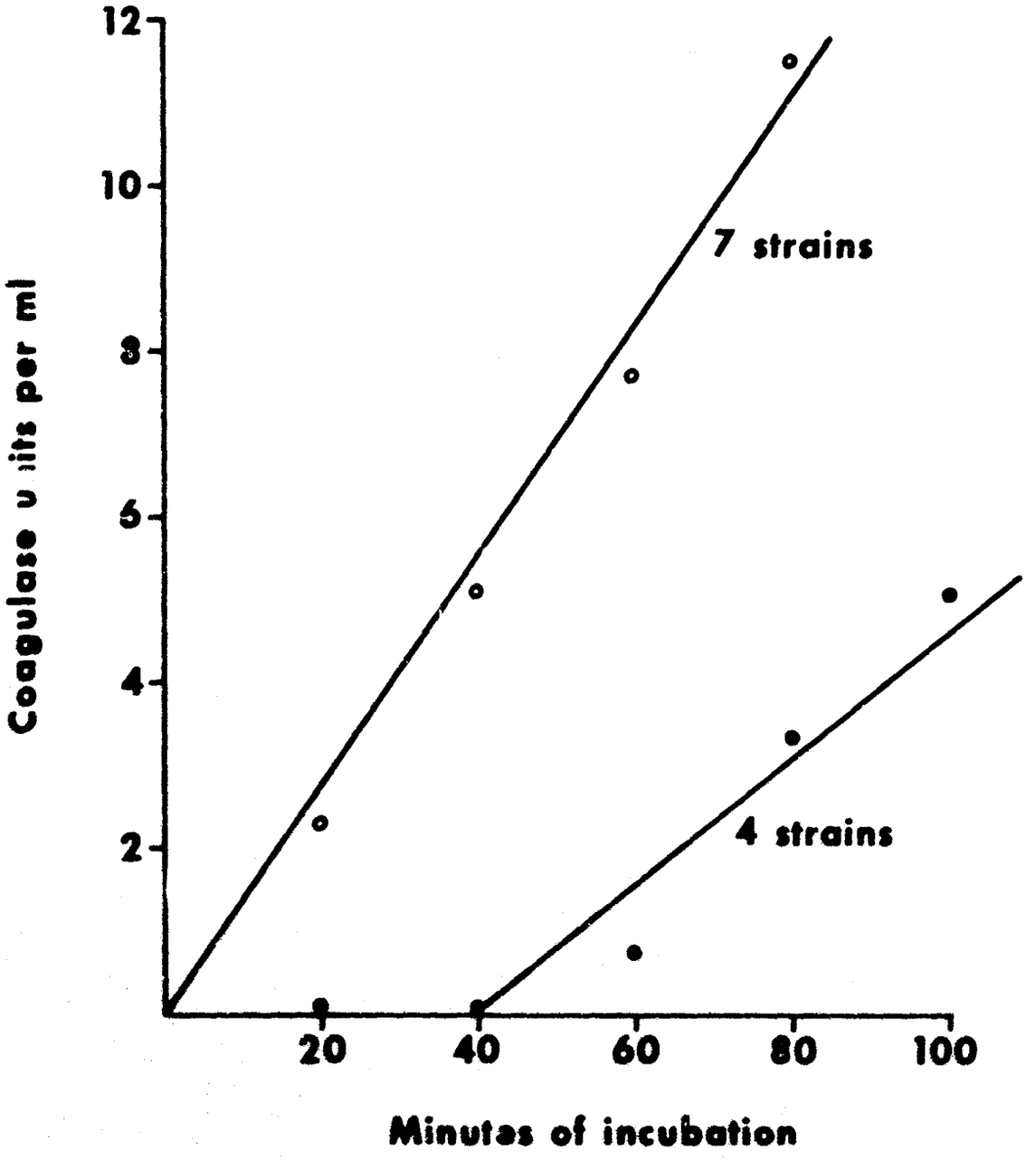


Figure 2. Variable Coagulase Release of Staphylococcus aureus Strains after Resuspension in Albumin Broth.

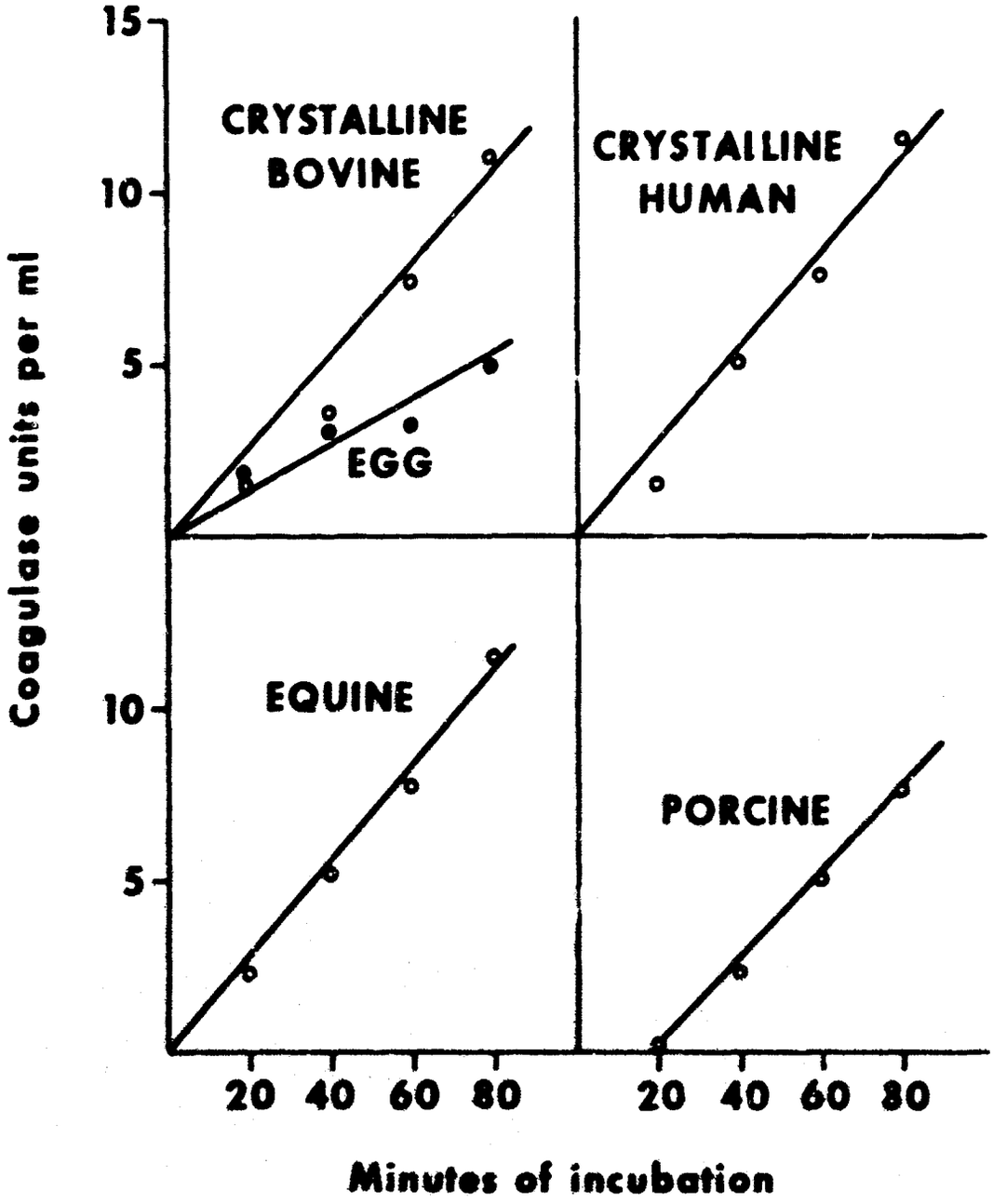


Figure 3. Promotion of Coagulase Release by 0.3% of Various Albumins (Haybush Strain).

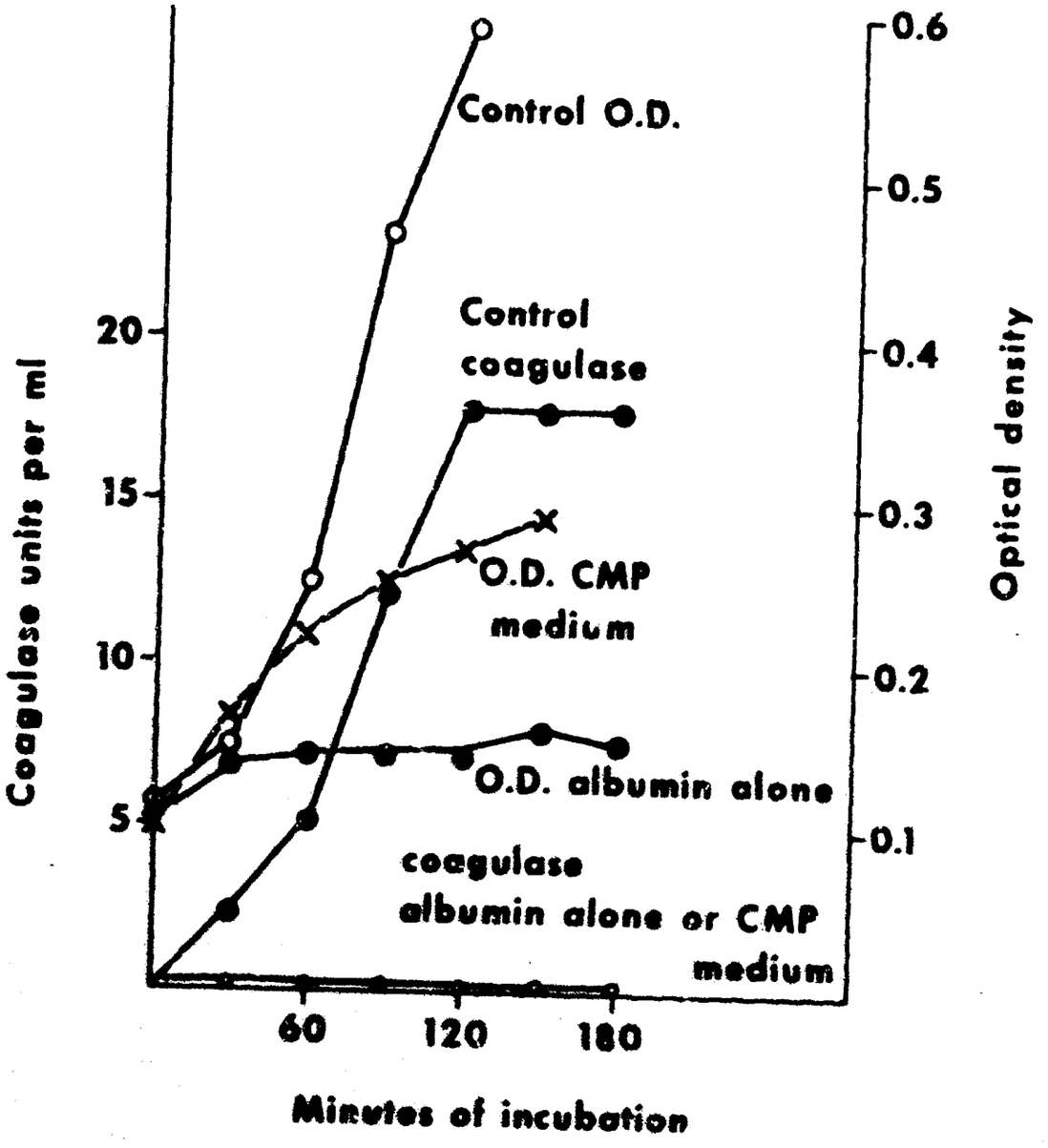


Figure 4. Effects of Chloramphenicol (CMP) Medium or Albumin Alone on Coagulase Production by Staphylococcus aureus (Smybush Strain).

TABLE 1. DIFFERENTIAL CHLORAMPHENICOL (CMP) INHIBITION OF COAGULASE RELEASE RATE AND CELL GROWTH RATE OF STAPHYLOCOCCUS AUREUS

| | Inhibition, % | | | |
|---|---|---|---|---|
| | Maybush Strain | | Smith Strain | |
| µg CMP/ml | Growth | Coagulase Release | Growth | Coagulase Release |
| 0.1 | 0 | 0 | 0 | 58 |
| 0.2 | 0 | 35 | 0 | 59 |
| 0.5 | 0 | 62 | 6.0 | 93 |
| 1.0 | 9.2 | 100 | 24.4 | 100 |

organism. In these experiments, chloramphenicol was added to the cultures in albumin-free medium 40 minutes before centrifugation and resuspension in medium containing albumin. Preliminary experiments had shown that, after addition of chloramphenicol, 40 minutes of incubation were required to establish the new steady state growth rate. Therefore, it appears that coagulase synthesis is much more sensitive to chloramphenicol than is general protein synthesis and cell growth.

It was noted in earlier studies that cultures in which albumin had been added to the medium prior to inoculation with a small number of cells stopped coagulase release after the optical density had reached 0.4 to 0.5, although cell growth continued unabated (Fig. 5). It was thought probable that some condition of the medium arising as a result of growth was suppressing coagulase release. Therefore, cultures were grown in albumin medium to the optical density at which coagulase release had stopped. The cells were then removed by centrifugation and the supernatant fluid was used to suspend cells freshly grown in trypticase soy broth to an optical density of 0.2. During subsequent incubation, nearly normal rate of growth of the new cells ensued but there was no further coagulase release, thus demonstrating that the used medium was preventing coagulase production. Careful measurements revealed that when coagulase released ceased, the pH had dropped to 6.3. Therefore, various batches of trypticase soy broth containing 0.3% bovine albumin were prepared and adjusted to varying pH values. These media were used to resuspend cells freshly grown in normal trypticase soy broth and the resulting suspensions were reincubated at 37 C. The results presented in Table 2 show that coagulase release is indeed quite sensitive to the pH of the medium, whereas growth rate seems to be unaltered by such relatively minor changes in pH.

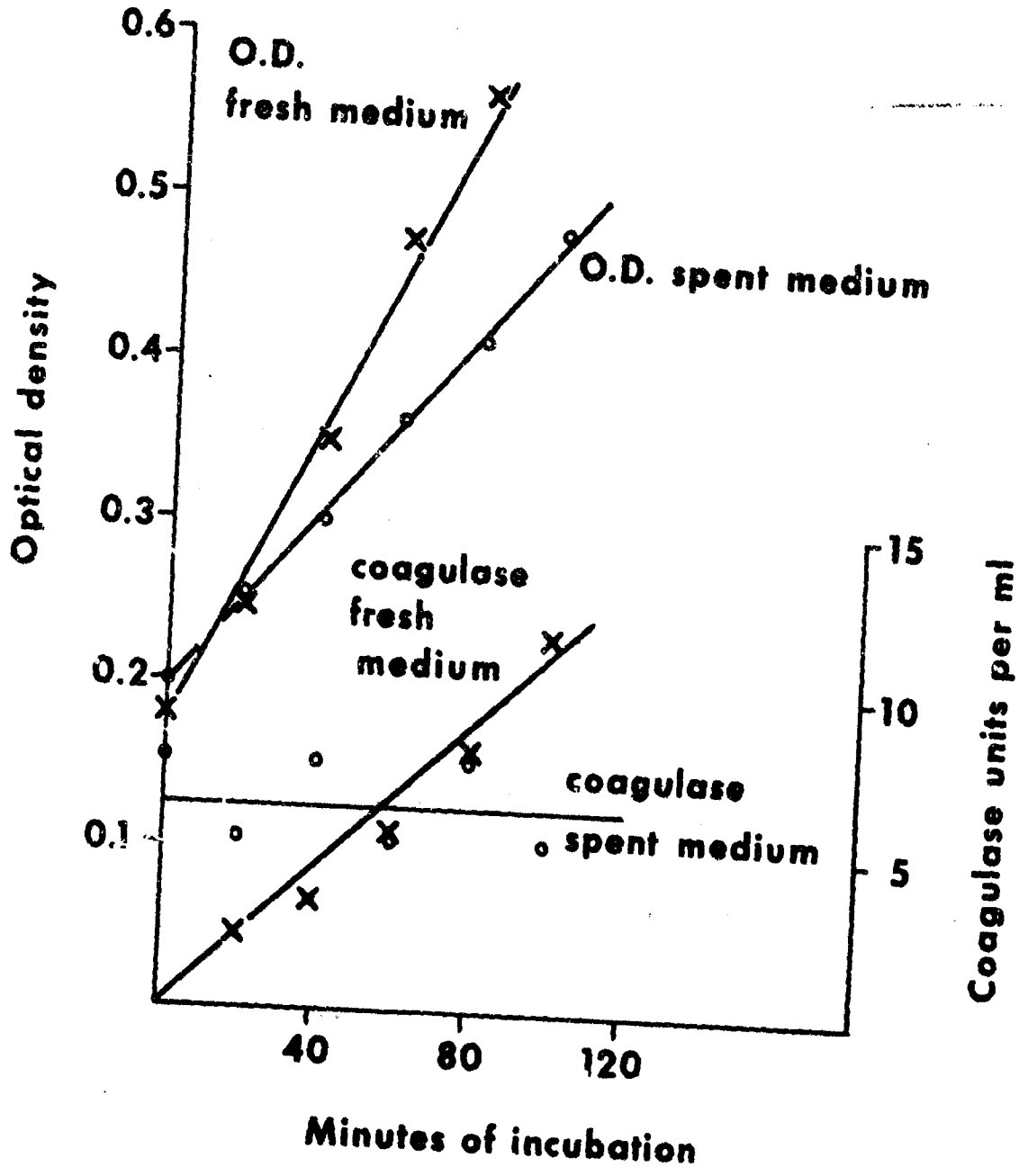


Figure 5. Growth and Coagulase Production of Staphylococcus aureus Cells Resuspended in Fresh or Spent Medium.

TABLE 2. SUPPRESSION OF COAGULASE RELEASE DURING GROWTH[a] OF *STAPHYLOCOCCUS AUREUS* IN MEDIA OF LOW pH

| pH of Medium | Units of Coagulase/ml | O.D. Increase |
|---|---|---|
| 6.3 | 0 | 0.32 |
| 6.5 | 3.4 | 0.33 |
| 6.9 | 7.7 | 0.34 |
| 7.3 | 7.7 | 0.33 |

a. Incubated at 37 C for 80 min.

As a final note it should be mentioned that all attempts to demonstrate coagulase in extracts of cells grown either with or without albumin in the medium have given negative results. Cells have been disrupted either by grinding with alumina or by destruction with the Branson sonifier. Results to date indicate that if there is any preformed, intracellular coagulase it must be present in an amount smaller than 1 unit per 65 μg of intracellular protein. Currently, our opinion is that staphylocoagulase is probable a surface-localized enzyme and that the normal fixation of the enzyme to the cell surface is antagonized by albumin, allowing coagulase to be solubilized and discharged into the medium.

Unclassified
Security Classification

**DOCUMENT CONTROL DATA - R&D**

*(Security classification of title, body of abstract and indexing annotation must be entered when the overall report is classified)*

| | |
|---|---|
| 1. ORIGINATING ACTIVITY (Corporate author)<br>U.S. Army Biological Laboratories<br>Fort Detrick, Frederick, Maryland, 21701 | 2a. REPORT SECURITY CLASSIFICATION<br>Unclassified<br>2b. GROUP |

3. REPORT TITLE

SOME CHARACTERISTICS AND KINETICS OF COAGULASE RELEASE BY STAPHYLOCOCCUS AUREUS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

5. AUTHOR(S) (Last name, first name, initial)

Altenbern, Robert A.

| 6. REPORT DATE | 7a. TOTAL NO. OF PAGES | 7b. NO. OF REFS |
|---|---|---|
| January 1966 | 16 | 2 |

| 8a. CONTRACT OR GRANT NO. | 9a. ORIGINATOR'S REPORT NUMBER(S) |
|---|---|
| b. PROJECT NO.<br>c. 1C014501B71A01<br>d. | Technical Manuscript 256<br>9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) |

10. AVAILABILITY/LIMITATION NOTICES

Qualified requestors may obtain copies of this publication from DDC. Foreign announcement and dissemination of this publication by DDC is not authorized. Release or announcement to the public is not authorized.

| 11. SUPPLEMENTARY NOTES | 12. SPONSORING MILITARY ACTIVITY |
|---|---|
| | U.S. Army Biological Laboratories<br>Fort Detrick, Frederick, Maryland, 21701 |

13. ABSTRACT

Coagulase is released from growing cells of strains of Staphylococcus aureus when purified bovine serum albumin is added to the medium. When cells are grown in albumin-free medium to an optical density of 0.2 and then resuspended in media containing albumin, two types of responses are noted: some strains show an immediate and steady rate of release of coagulase, others show a considerable (40 to 60 min) lag followed by release of coagulase at a much lower but also steady rate. Coagulase is not released by resuspension in albumin solution alone. Horse, human, bovine, and porcine albumins elicit the same rate of coagulase release, but egg albumin promotes coagulase release at a considerably lower rate. Bovine hemoglobin, gelatin, transferrin, and bovine alpha, beta, and gamma globulins do not promote the release of coagulase. Slightly acid pH suppresses release of coagulase from cells growing in media containing albumin. Proper concentrations of chloramphenicol completely prevent elaboration of coagulase but allow unaltered cell growth. Cell-free extracts prepared by two methods failed to show any intracellular coagulase.

DD FORM 1 JAN 64 1473

Unclassified
Security Classification